

Listing of Claims

What is claimed is:

1. (Currently amended) A nucleic acid sequence comprising:

Px-Sx-Bn-(ZR)-Hir(AsmR)-protein(Y)-T

where

Px is a promoter sequence;

Sx is a nucleic acid encoding a signal sequence or leader sequence;

Bn is 1-15 codons, when n is an integer from 1 to 15, or a chemical bond, when n=0;

Z is a codon for lysine or arginine;

R is an arginine codon or a chemical bond;

Hir is a nucleic acid sequence coding for hirudin ~~or a hirudin derivative which is at least about 80% homologous thereto;~~

ASm is a chemical bond, when m=O, or 1-10 codons, when m is an integer from 1 to 10;

protein(Y) is a nucleic acid sequence encoding mini-proinsulin ~~or a derivative thereof which is at least about 90% homologous thereto;~~ and

T is an untranslated expression-enhancing nucleic acid sequence.

Claims 2 to 6 (Canceled)

7. (Original) A multicopy vector comprising the nucleic acid of claim 1.

8. (Original) A plasmid comprising the nucleic acid of claim 1.

9. (Original) A host cell comprising the nucleic acid of claim 1, as part of the host cell chromosome, as part of a mini-chromosome, or extra-chromosomally.

10. (Original) The host cell of claim 9, wherein the host cell is a yeast.

11. (Original) The host cell of claim 10, wherein the yeast is selected from *Saccharomyces cerevisiae*, *Kluyveromyces lactis*, *Hansenula polymorpha*, and *Pichia pastoris*.

12. (Original) A host cell comprising the multicopy vector of claim 7.

13. (Original) A host cell comprising the plasmid of claim 8.

14. (Previously Presented) A process of fermentative production of fusion protein comprising expressing the nucleic acid of the host cell of claim 9 to form the fusion protein in a fermentation supernatant of a cell culture.

Claims 15 to 20 (Canceled)

21. (Currently amended) The process of claim 14 further comprising ~~further the step of~~ isolating said fusion protein from said fermentation supernatant.

22. (Currently amended) The process of Claim 21 wherein said ~~step of~~ isolating the fusion protein comprises adjusting the pH of said fermentation supernatant to about 2.5 to 3.5 to precipitate non-desired proteins and form a precipitation supernatant and isolating the fusion protein from said precipitation supernatant.

23. (Currently amended) The process of claim 14 further comprising ~~the steps of~~: (A) separating the fermentation supernatant from the host cell; (B) culturing the host cell in fresh medium; (C) separating the resulting supernatant from the host cell; (D) repeating ~~steps~~ (B) and (C) several times; and (E) isolating the fusion protein from the aforementioned supernatants by adjusting the pH of said supernatants to about 2.5 to 3.5 to precipitate non-desired proteins and form a precipitation supernatant and isolating the fusion protein from said precipitation supernatant.

24. (Currently amended) The process of claim 21, wherein the ~~step of~~ isolating the fusion protein comprises precipitating the fusion protein from the fermentation supernatant, and further comprising ~~the additional steps of~~ releasing the protein encoded by protein(Y) from the fusion protein and concentrating said protein encoded by protein(Y) by microfiltration, hydrophobic interaction chromatography, ion exchange chromatography, or a combination of such procedures.

25. (Previously Presented) A process of fermentative production of fusion protein, comprising expressing the nucleic acid of the host cell of claim 12 to form the fusion protein in a supernatant of a cell culture.

26. (Currently amended) The process of claim 25 further comprising ~~further the step of~~ isolating the fusion protein from the supernatant of the cell culture.

27. (Previously Presented) A process of fermentative production of fusion protein, comprising expressing the nucleic acid of the host cell of claim 13 to form the fusion protein in a supernatant of a cell culture.

28. (Currently amended) The process of claim 27 further comprising ~~further the step of~~ isolating the fusion protein from the supernatant of the cell culture.

29. (Canceled)

30. (Currently amended) A process of claim 21 further comprising ~~the step of~~ releasing mini-proinsulin by treating said fusion protein with trypsin and carboxypeptidase B.

31. (Currently amended) A nucleic acid sequence of claim 1 ~~2~~ in which (AsmR), taken together, is an arginine codon.

32. (Currently amended) A nucleic acid sequence of claim 1 ~~2~~ in which (AsmR), taken together, encodes SEQ ID NO: 12 (Gly-Asn-Ser-Ala-Arg).

33. (Currently amended) A nucleic acid sequence of claim 1 ~~2~~ in which Px is a yeast ADH2 promoter, Sx is an a factor leader sequence, and Hir encodes hirudin or lepirudin, (AsmR), taken together, is either an arginine codon or encodes SEQ ID NO:12 (Gly-Asn-Ser-Ala-Arg}, ~~and T is the 3' segment of the sequence coding for bovine interleukin 2 which remains after cleavage thereof with NotI restriction enzyme.~~

34. (Currently amended) A nucleic acid sequence of Claim 33 wherein protein(Y) is a nucleic acid sequence encoding mini-proinsulin.

35. (Currently amended) A nucleic acid of Claim 34 wherein Hir is a nucleic acid sequence encoding lepirudin which has been prepared recombinantly.